Plant Cell Physiol. 46(10): 1666–1673 (2005)
doi:10.1093/pcp/pci182, available online at www.pcp.oupjournals.org

Analysis of the Transport Activity of Barley Sucrose Transporter HvSUT1

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Localization studies indicate that barley (*Hordeum vulgare*) sucrose transporter HvSUT1 functions in sucrose uptake into seeds during grain filling. To further understand the physiological function of HvSUT1, we have expressed the HvSUT1 cDNA in *Xenopus laevis* oocytes and analyzed the transport activity by two-electrode voltage clamping. Consistent with a H^+-coupled transport mechanism, sucrose induced large inward currents in HvSUT1-expressing oocytes with a $K_{0.5}$ of 3.8 mM at pH 5.0 and a membrane potential of –157 mV. Of 21 other sugars tested, four glucosides were also transported by HvSUT1. These glucosides were maltose, salicin (2-(hydroxymethyl) phenyl β-D-glucoside), α-phenylglucoside and α-paranitrophenylglucoside. Kinetic analysis of transport of these substrates by HvSUT1 was performed and $K_{0.5}$ values were measured. The apparent affinity for all substrates was dependent on membrane potential and pH with lower $K_{0.5}$ values at lower external pH and more negative membrane potentials. HvSUT1 was more selective for α-glucosides over β-glucosides than the *Arabidopsis* sucrose transporter AtSUC2. Several substrates transported by AtSUC2 (β-phenylglucoside, β-paranitrophenylglucoside, α-methyl-glucoside, turanose, and arbutin (hydroquinone β-D-glucoside)) showed low or undetectable transport by HvSUT1. Of these, β-paranitrophenylglucoside inhibited sucrose transport by HvSUT1 indicating that it interacts with the transporter while arbutin and α-methyl glucoside did not inhibit. The results demonstrate significant differences in substrate specificity between HvSUT1 and AtSUC2.

Keywords: Electrophysiology — *Hordeum vulgare* — HvSUT1 — Oocyte expression — Sucrose transporter.

Introduction

Plant sucrose transporters (SUTs or SUCs) have two main functions in the long-distance transport of sucrose. In photosynthetic leaves (and other source tissue), they function in sucrose uptake into companion cells and sieve elements of the phloem (vascular tissue) in a process called phloem loading. In sink tissue such as roots, developing leaves, flowers, and seeds, that depend on imported sugar, sucrose transporters function in post-phloem sucrose uptake into sink cells (Patrick and Offler 2001, Truernit 2001). Dicots encode three types of sucrose transporters (Fig. 1). In *Arabidopsis*, the Type I transporter AtSUC2 has a higher affinity for sucrose (Sauer and Stolz 1994, Chandran et al. 2003) compared to the Type II transporter AtSUT2/SUC3 (Meyer et al. 2000, Schulze et al. 2000), and the Type III transporter AtSUT4 (Weise et al. 2000). However, a detailed analysis of substrate specificity has only been conducted for AtSUC2 (Chandran et al. 2003) and so the three types of sucrose transporters cannot be compared yet on that basis. It is also clear that in monocots, such as rice, maize, and barley, only Type II and III sucrose transporters are present (Aoki et al. 2003). In dicots, sucrose transporters related to AtSUC2 are required for sucrose loading into phloem in source leaves. For example, insertional mutants in AtSUC2 are conditionally lethal (Gottwald et al. 2000). Therefore, it is surprising that monocots do not contain Type I sucrose transporters and apparently use SUTs of a different type for phloem loading. In rice, five SUT homologs have been identified (Fig. 1) and it is not clear which of these contribute to phloem loading. As shown by reverse transcriptase PCR (RT-PCR), all five SUT genes in rice are expressed in tissues throughout the plant including source leaves, developing leaves and seeds (Aoki et al. 2003). Antisense inhibition of OsSUT1 did not alter carbohydrate export from leaves (Ishimaru et al. 2001) indicating that OsSUT1 does not have a unique function in phloem loading.

Of the five rice sucrose transporter genes, only OsSUT1 expression is correlated with grain filling (Aoki et al. 2003). During grain filling, transmembrane sucrose transport is important for the delivery of sucrose from maternal tissues to support the synthesis of starch and other storage compounds in the endosperm of the seed. Antisense inhibition of OsSUT1 from rice resulted in aborted endosperm tissue (Scofield et al. 2002) providing strong evidence that OsSUT1 has a major function in the uptake of sucrose by developing seeds. The cellular location of OsSUT1 expression in rice seeds has not been investigated; however, in barley the closely related sucrose transporter HvSUT1 is expressed in endosperm transfer cells and, similarly to OsSUT1, expression is developmentally correlated with grain filling (Weschke et al. 2000). Endosperm transfer cells are specialized for transmembrane transport and represent the main interface for assimilate exchange between maternal and filial tissue. These results indicate that OsSUT1 in rice and HvSUT1 in barley have important functions in sucrose uptake into seeds. Despite the potential importance of the monocot SUT1 transporters, little is known concerning their transport activity. In addition, only limited information exists on the

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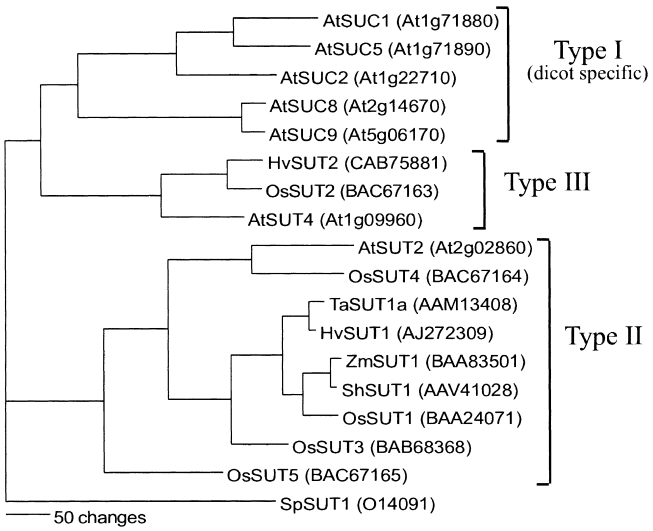


Fig. 1 Phylogenetic tree of selected sucrose transporters. Protein sequences were aligned using Clustal X and the neighbor-joining tree was made using PAUP 4.0. Gene names (for *Arabidopsis* transporters) or Genbank accession numbers are provided. The tree was rooted using the SpSUT1 sequence from *S. pombe* (Reinders and Ward 2001). Assignments of Type I, II and III were made according to Aoki et al. (2003).

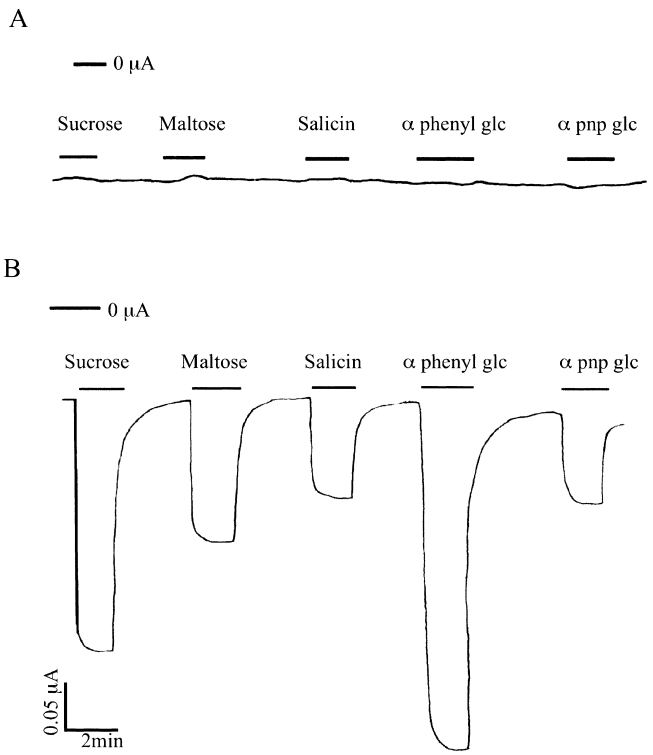


Fig. 2 Sugars transported by HvSUT1. *Xenopus* oocytes, either uninjected (A) or injected with HvSUT1 cRNA (B), were voltage clamped and currents were recorded. The membrane potential was held at –40 mV in sodium Ringer solution at pH 5.25. Substrates were applied at 10 mM concentrations in the same solution at the times indicated and downward deflections indicate inward proton (coupling ion) current. Abbreviations: α pnp glc = α-paranitophenylglucoside; α phenyl glc = α-phenylglucoside.

transport properties of Type II sucrose transporters in dicots (Meyer et al. 2000, Schulze et al. 2000).

The substrate specificities of sucrose transporters outside the Type I subfamily have not been studied in detail. This is especially true for sucrose transporters from monocots for which information is limited to the following: OsSUT1 and 3 from rice have been shown to allow yeast strain SuSy7 (Riesmeier et al. 1992) to grow on sucrose (Aoki et al. 2003). Sucrose transporters from barley, HvSUT1 and 2, were expressed in yeast and K_m values for sucrose transport of 7.5 mM and 5 mM were reported (Weschke et al. 2000). ZmSUT1 from maize was expressed in *Xenopus* oocytes, the K_m for sucrose and H^+ was shown to be pH and voltage dependent, and H^+-coupled sucrose transport was demonstrated to be reversible (Carpaneto et al. 2005).

This study was initiated to improve our understanding of factors controlling sucrose uptake into monocot seeds. The HvSUT1 cDNA was expressed in *Xenopus laevis* oocytes and transport properties were analyzed by voltage clamping. The results show that compared to Type I sucrose transporters, HvSUT1 has a lower affinity for sucrose but is more selective for sucrose over other substrates.

Results

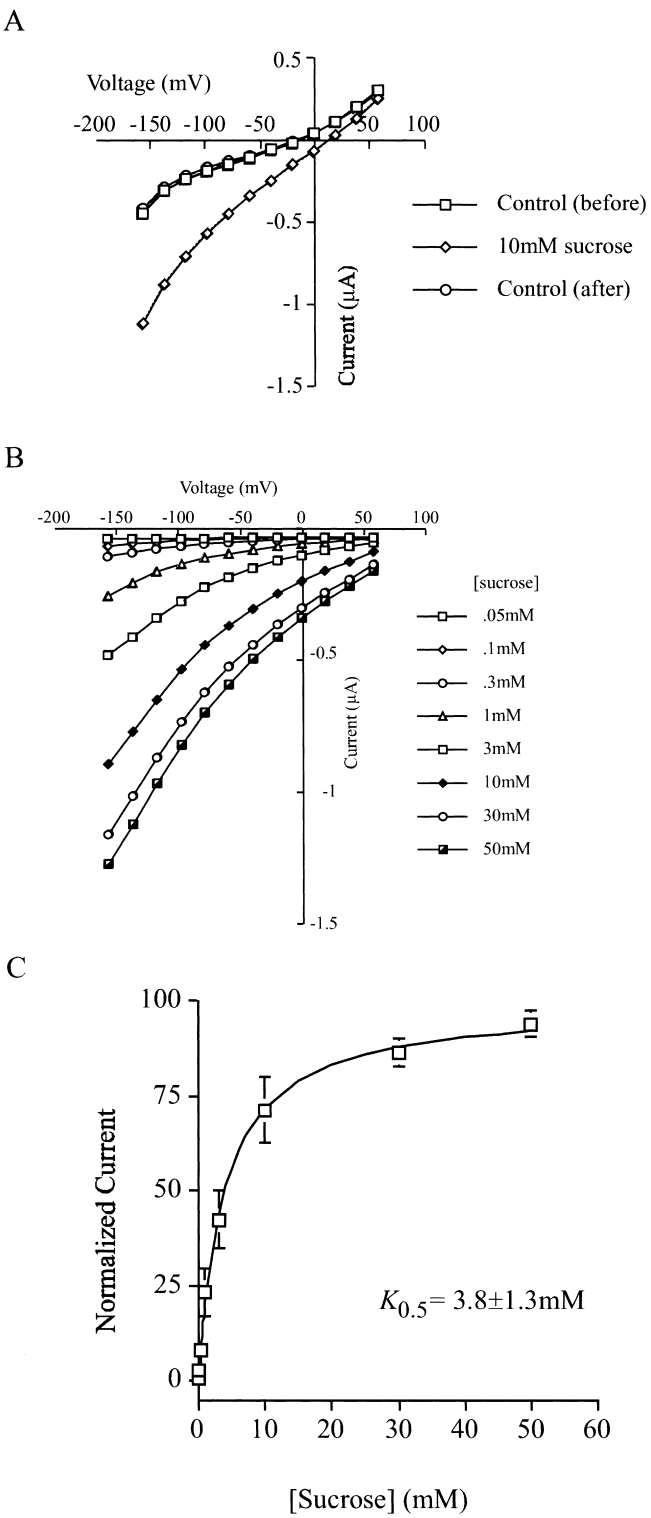
Sucrose and other glucosides induced large inward currents in HvSUT1-expressing oocytes

Two-electrode voltage clamping was used to investigate the substrate specificity and other transport properties of the barley sucrose transporter HvSUT1. Sugars, at 10 mM in sodium Ringer solution at pH 5.25, were applied to voltage clamped oocytes and, as shown in Fig. 2B, large inward currents were induced by 10 mM sucrose, maltose, salicin, α-phenylglucoside, and α-paranitrophenylglucoside. The average sucrose-induced current under the same conditions at the holding potential of –40 mV was 210 ± 70 nA (n = 4 oocytes). The observed inward currents, induced by uncharged substrates, were consistent with a proton-coupled mechanism for the plant sucrose transporters (Boorer et al. 1996). When Na^+ in the bath solution was replaced with 115 mM K^+, sucrose-induced currents were identical, indicating that transport was not Na^+ coupled. None of the sugars tested induced significant currents in uninjected oocytes (Fig. 2A).

Kinetic analysis of sucrose transport by HvSUT1

The current-voltage relation was analyzed in HvSUT1-expressing oocytes before, during and after applications of sucrose (Fig. 3A). Currents were measured at potentials from –157 mV to 57 mV. Typical results for currents induced by 10 mM sucrose at pH 5.0 are presented in Fig. 3A. Sucrose-

dependent currents at various sucrose concentrations were obtained by subtraction of an average of baseline currents before and after sucrose application (Fig. 3B). Sucrose-dependent currents were slightly more voltage dependent (activation at more negative potentials) than previously characterized high affinity plant sucrose transporters StSUT1 (Boorer et al. 1996), AtSUC1 (Zhou et al. 1997) and AtSUC2 (Chandran et al. 2003). Sucrose-dependent currents did not reverse under the experimental conditions used (up to 57 mV; Fig. 3B), consistent with a lack of substrate within the oocyte cytoplasm. Experiments were performed with sucrose concentrations from 0.05 mM to 50 mM (Fig. 3B) and currents fitted to the Michaelis-Menten equation. The apparent affinity ($K_{0.5}$) for sucrose transport at pH 5.0 and at a membrane potential of –157 mV was 3.8 ± 1.3 mM (n = 5). Therefore, HvSUT1 can be classified as a moderate affinity sucrose transporter (see Discussion). V_{max} values varied between individual oocytes depending on time after RNA injection, therefore currents were normalized to V_{max} for presentation in Fig. 3C.



Sucrose uptake by a proton-coupled mechanism is driven by both the transmembrane pH gradient and the membrane potential. $K_{0.5}$ values were measured at a range of pH values and membrane potentials to determine if HvSUT1 was also regulated by these factors. Similar to high affinity sucrose transporters AtSUC1, AtSUC2, and potato (*Solanum tuberosum)* StSUT1 (Boorer et al. 1996, Zhou et al. 1997, Chandran et al. 2003), the apparent affinity of HvSUT1 was highly dependent on external pH and membrane potential (Fig. 4). At low pH (pH 5.0 and 5.3) the $K_{0.5}$ was low and voltage independent. At higher pH, $K_{0.5}$ values were voltage dependent with lower $K_{0.5}$ at more negative membrane potentials. At membrane potentials that approximated to those of plant cells (–157 mV; Fig. 4 inset) decreasing external pH lowered the $K_{0.5}$ for transport. These results indicate that increases in plasma membrane H^+-ATPase activity, which acidifies the extracellular space and hyperpolarizes the cell would stimulate sucrose uptake by increasing the apparent affinity for sucrose of HvSUT1.

HvSUT1 is selective for α-linked glucosides

To analyze the substrate specificity of HvSUT1, 22 different sugars were tested for their ability to induce currents in HvSUT1-expressing oocytes. Potential substrates were applied at pH 5.25 in sodium Ringer solution. From a holding potential of –40 mV, voltage pulses were applied from –157 mV to 57 mV as in Fig. 3A. Currents were normalized to sucrose-dependent currents to account for different expression levels. Average inward currents induced at –157 mV are presented in

Fig. 3 Kinetic analysis of sucrose transport by HvSUT1 expressed in *Xenopus* oocytes. Currents were recorded at pH 5.0. (A) Currents recorded before, during and after application of 10 mM sucrose. (B) Sucrose-dependent currents recorded at different sucrose concentrations. Background currents, before and after sucrose application (as in A) were averaged and subtracted from currents recorded during sucrose application. (C) Sucrose-dependent currents at a membrane potential of –157 mV (as in B) plotted against sucrose concentration. Line indicates a fit of the Michaelis-Menten equation to the data; error bars are SD (n = 5 oocytes).

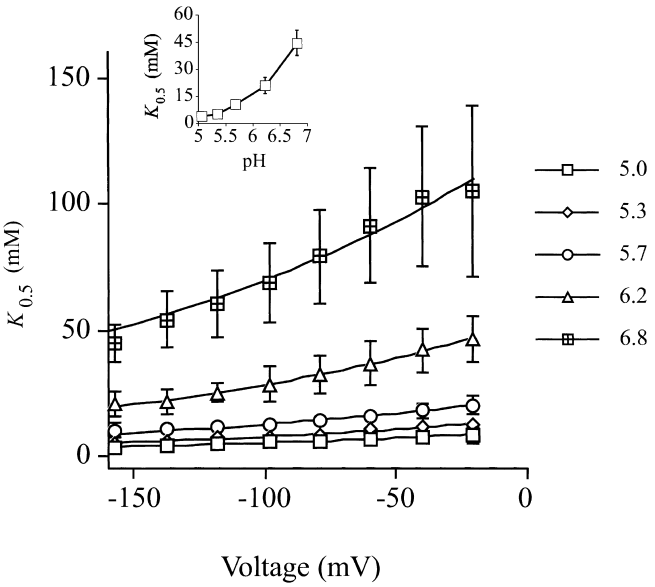


Fig. 4 Voltage-dependence of substrate apparent affinity for HvSUT1 at several pHs. $K_{0.5}$ values for sucrose were measured at concentrations between 50 μM and 50 mM. Mean $K_{0.5}$ values ± SD (n = 3 for pH 5.3, n = 4 for pH 5.7 and 6.8, n = 5 for pH 6.2 and 5.0) are plotted as a function of membrane potential. Data from –157mV to –20.5mV were fitted with Equation 1 (see Materials and Methods). Inset: pH dependence of $K_{0.5}$ at membrane potential –157 mV.

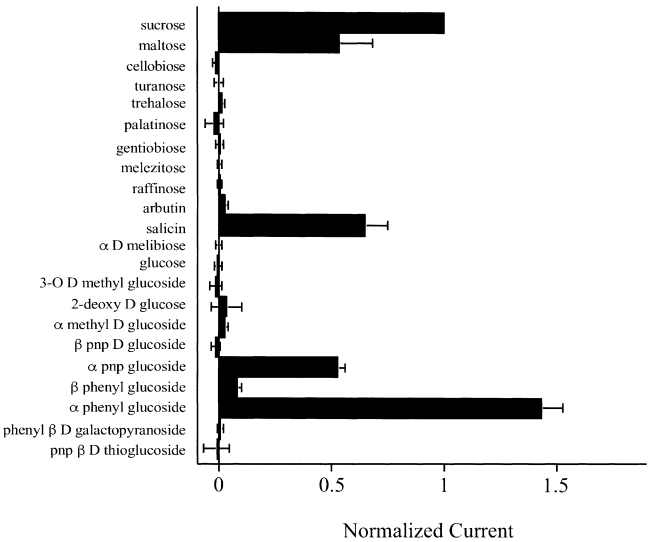


Fig. 5 Substrate specificity of HvSUT1. Substrate-dependent currents were recorded from *Xenopus* oocytes expressing HvSUT1 under voltage-clamp conditions. Substrates were applied at 10 mM in sodium Ringer solution, pH 5.25. Currents were recorded at a membrane potential of –157 mV. Substrate-dependent currents were normalized to currents recorded with 10 mM sucrose to control for differences in HvSUT1 expression between oocytes. Mean currents for 3 oocytes ± SE are presented.

Fig. 5. In addition to sucrose, maltose, salicin, α-paranitrophenylglucoside, and α-phenylglucoside induced large inward currents, indicating that they serve as transported substrates. HvSUT1 showed strong selectivity for α-glucosides over β-glucosides as clearly indicated by large currents induced by α phenylglucoside and α-paranitrophenylglucoside, while β-phenylglucoside and β-paranitrophenylglucoside induced small or undetectable currents, respectively. The only β-glucoside that induced significant currents was salicin, while the structurally related β-glucoside arbutin did not induce significant currents.

Kinetic analysis was performed for substrates that were found to induce large currents. Consistent with other high and low affinity sucrose transporters characterized to date (Weise et al. 2000, Chandran et al. 2003), the apparent affinity of HvSUT1 for maltose ($K_{0.5}$ = 14.9 ± 3.8 mM; Fig. 6A) was lower than for sucrose ($K_{0.5}$ = 3.8 ± 1.3 mM). This characteristic does not necessarily extend to homologs outside of the plant kingdom as the *Schizosaccaromyces pombe* homolog SpSUT1 transports maltose with a higher affinity (Reinders and Ward 2001). α-phenylglucoside showed a higher apparent affinity ($K_{0.5}$ = 2.2 ± 0.4 mM; Fig. 6C) than sucrose. This was also true for the high affinity sucrose transporter AtSUC2 (Chandran et al. 2003). However, HvSUT1 showed a lower apparent affinity for α-paranitrophenylglucoside ($K_{0.5}$ = 7.9 ± 3.1 mM; Fig. 6D) than for sucrose. In contrast, α-paranitrophenylglucoside was transported with the highest affinity of any substrate tested for AtSUC2 (Chandran et al. 2003). The apparent affinity of HvSUT1 for salicin was 7.3 ± 3.1 mM (Fig. 6B). This is of interest because salicin is a plant glucoside and although this represents a low affinity for salicin, it indicates that HvSUT1 could transport salicin or a related glucoside in plants in addition to sucrose and maltose.

$K_{0.5}$ values for five substrates were analyzed at a range of membrane potentials. The results in Fig. 7 were obtained at a pH (5.0) where the $K_{0.5}$ for sucrose is approximately voltage independent. The only substrate showing higher affinity for HvSUT1 than sucrose was α-phenylglucoside and $K_{0.5}$ values for α-phenylglucoside were voltage independent at pH 5 (Fig. 7). However, $K_{0.5}$ values for substrates with lower affinity were voltage dependent with $K_{0.5}$ increasing at depolarized membrane potentials. The results indicate that at low pH, HvSUT1 becomes less selective for sucrose over other substrates as the membrane potential becomes more negative. The voltage dependence of $K_{0.5}$ was similar for all transported substrates (Fig. 7, Table 1) except for α-paranitrophenylglucoside which showed much stronger voltage dependence as indicated by higher δ values (Table 1).

HvSUT1 interaction with non-transported substrates

Several glucosides transported by AtSUC2 (Chandran et al. 2003) were not transported by HvSUT1 (Fig. 5). The ability of arbutin, α-methyl glucoside, and β-paranitrophenylglucoside to inhibit sucrose-dependent inward currents was tested. Only β-paranitrophenylglucoside inhibited sucrose-dependent inward currents (Fig. 8A). This shows that β-paranitrophenyl-

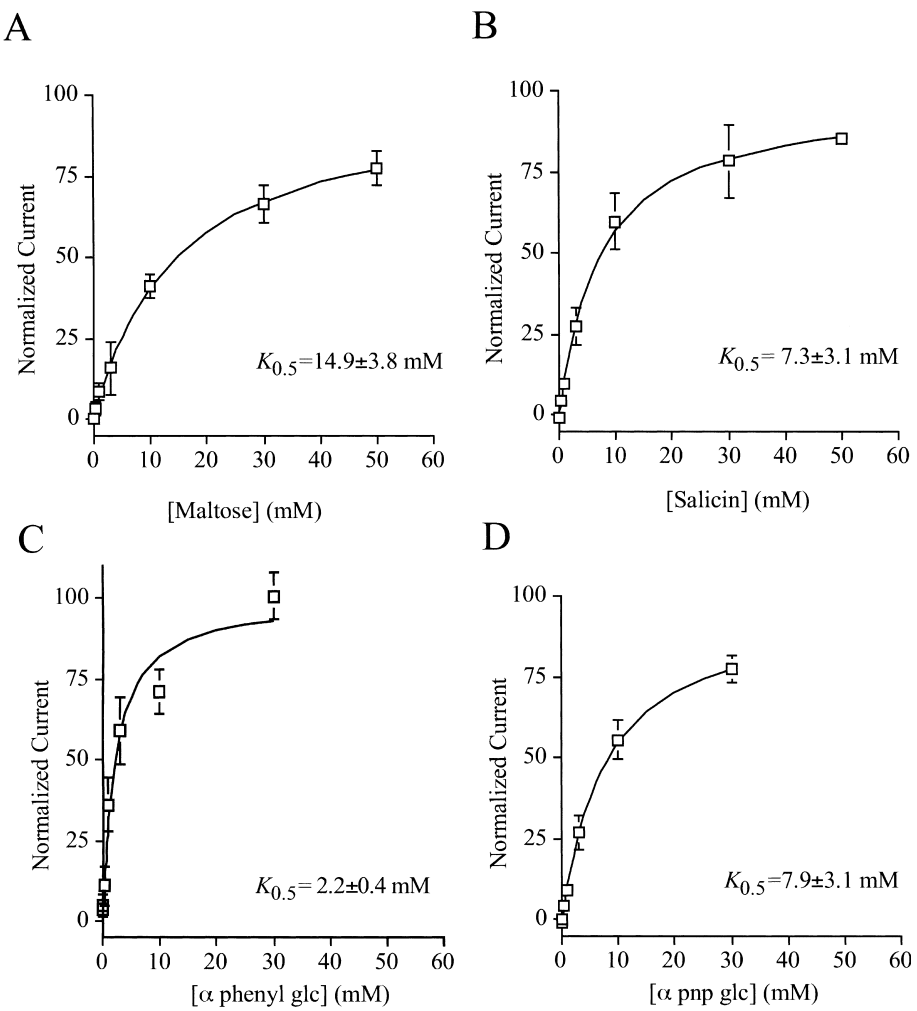


Fig. 6 Kinetic analysis of four transported substrates. Substrate-dependent currents were normalized to V_{max} and plotted against substrate concentration. Currents were measured at pH 5.0 and with a membrane potential of –157 mV. Line indicates a fit of the Michaelis-Menten equation to the data; error bars are SE (n = 3 oocytes). (A) maltose, (B) salicin, (C) α-phenylglucoside, and (D) α-paranitrophenylglucoside.

glucoside interacts with HvSUT1 leading to a block of the transporter. Inhibition by β-paranitrophenyl glucoside was voltage dependent, with greater inhibition at more negative membrane potentials (Fig. 8B).

Discussion

HvSUT1 transport activity and kinetics

HvSUT1 expression in oocytes resulted in large sucrose-induced inward currents, approximately 4-fold larger than AtSUC2 expressed from the same vector and recorded under the same conditions (Chandran et al. 2003) and also larger than other Type I SUTs that have been studied by oocyte expression (Boorer et al. 1996, Zhou et al. 1997). This high transport activity is of interest because previous studies in which other type II SUTs from dicots were expressed in yeast (Meyer et al. 2000, Schulze et al. 2000, Barth et al. 2003) showed transport rates in the range of 10 to 100-fold lower than AtSUC2 (Sauer and Stolz 1994) or StSUT1 from potato (Schulze et al. 2000). Low or non-detectable transport activity of LeSUT2 was suggested to be consistent with a function of this transporter as a sucrose sensor (Barker et al. 2000). Results presented here for expression of HvSUT1 in oocytes show a high transport rate and indicate that the low transport activity of dicot Type II SUTs, when expressed heterologously, is not a characteristic of all Type II SUTs.

HvSUT1 from barley showed a moderate apparent affinity for sucrose, having a $K_{0.5}$ of 3.8 ±1.3 mM at pH 5.0 and a membrane potential of –157 mV. A previous study of HvSUT1 using expression in yeast found that the $K_{0.5}$ for HvSUT1 at pH 6.0 was about 7.5 mM (Weschke et al. 2000), which is significantly lower than the $K_{0.5}$ reported here at this pH (approximately 16 mM). A potential cause for the systematic underestimation of $K_{0.5}$ values measured by ^{14}C sucrose uptake in yeast compared with voltage clamping in oocytes has been proposed (Chandran et al. 2003).

Voltage and pH dependence of the $K_{0.5}$ for transported substrates

The apparent affinity of HvSUT1 for sucrose was dependent on both pH and membrane potential. At low pH (< 6.0) $K_{0.5}$

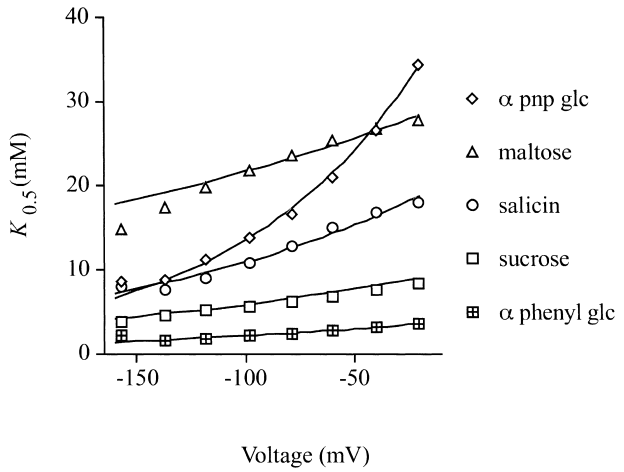


Fig. 7 Voltage-dependence of substrate apparent affinity for HvSUT1. $K_{0.5}$ values for transported substrates were measured at pH 5.0 for substrate concentrations between 50 μM and 50 mM (30 mM for α-phenylglucoside and α-paranitrophenylglucoside). $K_{0.5}$ values (mean for 3 oocytes; $n = 5$ for sucrose) are plotted as a function of membrane potential. Abbreviations for substrates are given in Fig. 2. Data from –157 mV to –20.5 mV were fitted with Equation 1 (see Materials and Methods). Error bars omitted for clarity. Data for the voltage dependence of sucrose are the same as data for pH 5 found in Fig. 4.

values were low and relatively voltage independent (Fig. 4). However, at higher pH $K_{0.5}$ values were voltage dependent with lower $K_{0.5}$ values at more negative potentials. These results are similar to reports for potato StSUT1 (Boorer et al. 1996) and *Arabidopsis* AtSUC1 (Zhou et al. 1997), both high affinity Type I transporters. To further explore the interaction between pH and membrane potential in determining apparent substrate affinity we tested the voltage dependence of $K_{0.5}$ values for four additional substrates (Fig. 7). These experiments were performed at pH 5.0. HvSUT1 had a lower $K_{0.5}$ for α-phenylglucoside than for sucrose and at pH 5.0 $K_{0.5}$ values were voltage independent for both α-phenylglucoside and sucrose. However, at this pH, $K_{0.5}$ values for substrates with lower apparent affinity than sucrose were significantly voltage dependent. This was especially true for α-paranitrophenylglucoside which showed a $K_{0.5}$ value similar to salicin at –157 mV and much higher $K_{0.5}$ values at less negative potentials. Since none of the glucosides used in this study are charged, the effects of membrane potential on $K_{0.5}$ values are not due to an influence of the electrical field on the glucoside substrate. The interaction between pH and membrane potential on $K_{0.5}$ suggests that one or more sites that can be protonated affect $K_{0.5}$ values. From the current results it is not possible to distinguish whether membrane potential-induced changes in apparent affinity are caused by protein conformation changes or through influencing coupling ion (H^+) interaction with the protein. The strong voltage dependence of $K_{0.5}$ values for α-paranitrophenylglucoside (Fig. 7) compared to that of other substrates may indicate α-paranitrophenylglucoside interacts with the transporter glucoside binding site differently than other substrates, for example the binding site for α-paranitrophenylglucoside may be extended compared to the binding site for other substrates.

The effects of external pH and membrane potential on $K_{0.5}$ values for different sugars could be important for regulating which sugars are transported into the endosperm of developing seeds. At more negative membrane potentials HvSUT1 is less selective for sucrose over maltose and salicin as demonstrated at an external pH of 5.0 (Fig. 7). At more depolarized potentials HvSUT1 became relatively more selective for sucrose.

Transport specificity

The major difference found for HvSUT1 compared to other characterized sucrose transporters was in substrate specificity. HvSUT1 was shown here to be more selective for sucrose than AtSUC2 from *Arabidopsis* (Chandran et al. 2003). Of the 22 sugars tested only five induced significant currents in oocytes expressing HvSUT1 (Fig. 5). The $K_{0.5}$ value for sucrose was lower than for maltose. This characteristic is shared with all other plant sucrose transporters but does not extend to the homologous transporter from *S. pombe*, SpSUT1, which has a higher affinity for maltose over sucrose (Reinders

Table 1 Summary of $K_{0.5}$ and δ values for substrates transported by HvSUT1

Substrate	$K_{0.5}$ (mM) at –157mV	δ (%)
α-phenylglucoside (pH 5.0)	2.2 ± 0.4	15.9 ± 1.2
Salicin (pH 5.0)	7.3 ± 3.1	17.4 ± 1.4
α-paranitrophenylglucoside (pH 5.0)	7.9 ± 3.1	29.9 ± 2.3
Maltose (pH 5.0)	14.9 ± 3.8	8.6 ± 1.1
Sucrose (pH 5.0)	3.8 ± 1.3	13.9 ± 8.5
Sucrose (pH 5.3)	5.17 ± 0.5	15.9 ± 8.5
Sucrose (pH 5.7)	10.6 ± 2.6	16.6 ± 8.4
Sucrose (pH 6.2)	21.1 ± 4.5	14.8 ± 4.2
Sucrose (pH 6.8)	44.6 ± 7.0	14.5 ± 4.2

δ values, a measure of voltage dependence of $K_{0.5}$, are presented as a percentage of the membrane electric field. pH of the recording solution is shown in parentheses.

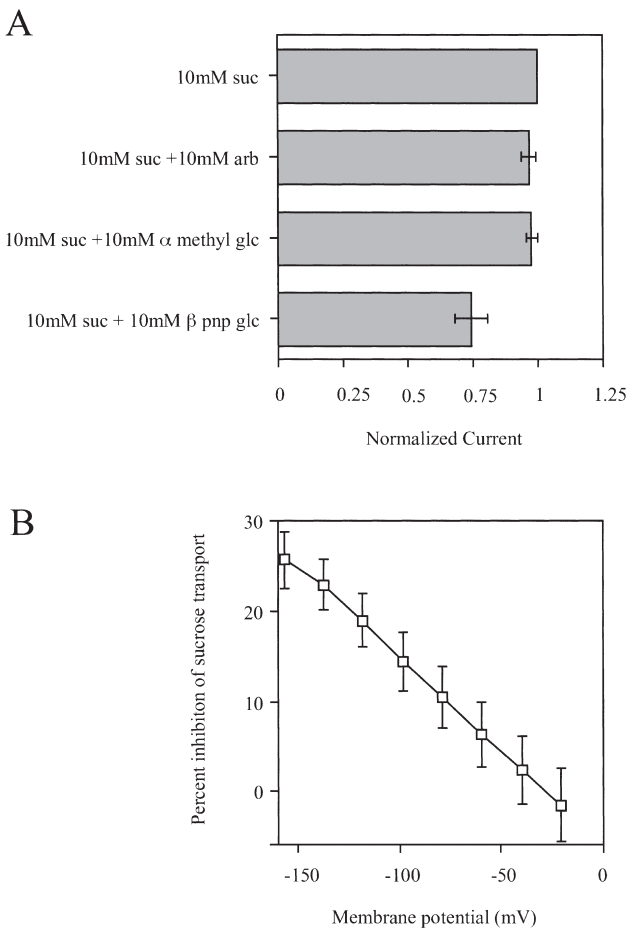


Fig. 8 Analysis of inhibition of sucrose transport by non-transported glucosides. Oocytes expressing HvSUT1 were bathed in sodium Ringer solution at pH 5.0 and voltage clamped. (A) Currents were recorded in the presence of 10 mM sucrose with and without 10 mM concentrations of competing glucosides as indicated at a membrane potential of –157 mV and normalized to sucrose-dependent currents. Mean currents for 5 oocytes ± SE are presented. (B) Voltage dependence of inhibition of sucrose transport by β-paranitrophenylglucoside. Currents were recorded and normalized as in A, at membrane potentials as indicated. Data are mean percent inhibition for 5 oocytes ± SE.

and Ward 2001). HvSUT1 showed an extreme selectivity for α-phenylglucosides over β-phenylglucosides (Fig. 5), a characteristic that was not observed for AtSUC2 (Chandran et al. 2003). However, HvSUT1 did transport the plant β-linked glucoside salicin but not the closely related β-linked glucoside arbutin. In contrast, AtSUC2 transports both salicin and arbutin with high affinity (Chandran et al. 2003). These results indicate that HvSUT1 selectivity is not based on differentiating glucoside linkage orientation, but rather is determined by the combination of side chain structure and glucoside linkage.

HvSUT1 did not transport α-methyl glucose (Fig. 5) while AtSUC2 showed significant transport of this substrate (Chandran et al. 2003). Hitz et al. (1986) proposed that the fructosyl moiety of sucrose does not interact specifically with the transporter but presents a hydrophobic surface that interacts with the binding site, while the glucosyl hydroxyls 3, 4, and 6 interact directly with the binding site. Therefore it was of interest that AtSUC2 could transport glucose with an O-linked methyl group at the 1 but not the 3 position. This characteristic does not extend to HvSUT1, indicating that differences in the binding sites between HvSUT1 and AtSUC2 are evident for even the smallest substrates.

The inhibition of HvSUT1 by β-paranitrophenylglucoside (Fig. 8) indicates either an interaction at the substrate binding site that does not result in transport or the presence of another regulatory binding site for glucosides. Many previous studies using ^{14}C-sucrose uptake in yeast showed inhibition of sucrose uptake by glucosides that have subsequently been shown to be transported substrates (Chandran et al. 2003). This is the first demonstration of a glucoside that inhibits transport activity. It is possible that inhibitory glucosides also exist in plants and regulate sucrose transport.

In conclusion, the substrate specificity of HvSUT1, which functions in sucrose uptake into seeds in the monocot barley is significantly different from that of AtSUC2 which functions in loading sucrose into the phloem in the dicot *Arabidopsis*. Further work will be required to determine if differences between HvSUT1 and AtSUC2 presented here represent differences between Type I and Type II SUTs. It is also possible that monocot and dicot sucrose transporters have differences in substrate specificity and it would be interesting to compare the activity of HvSUT1 to a Type II SUT from a dicot, such as *Arabidopsis* AtSUT2. The final possibility is that sucrose transporters functioning in sink tissue, such as HvSUT1 in seeds, are generally more selective than transporters responsible for phloem loading, such as AtSUC2. This would be of interest in understanding how plants control which sugars are accumulated in sinks such as seeds.

Materials and Methods

Subcloning

The barley (*Hordeum vulgare*) HvSUT1 coding region was excised from pBK-CMV-HvSUT1 (Weschke et al. 2000) with SpeI and XhoI and directionally subcloned into the oocyte expression vector pOO2 (Ludewig et al. 2002). Sequencing this construct and the pBK-CMV-HvSUT1 clone revealed four nucleotide differences resulting in two amino acid changes compared to the original report (Weschke et al. 2000; Genbank accession AJ272309). The updated sequence has been deposited in Genbank (AM055812). This construct was linearized using PmaCI (PanVera, Madison, WI, USA) and 1 μg was used as template for cRNA synthesis using the mMessage mMachine kit (Ambion, Austin, TX, USA).

Heterologous expression

Stage V and VI *Xenopus* oocytes, were incubated in Barth's medium (88mM NaCl, 1mM KCl, 0.33mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 10 mM HEPES, pH 7.6, 100 μg ml^{-1} penicillin and 100 μg ml^{-1} streptomycin) containing 10 mg ml^{-1} collagenase A (Roche Applied Science, Indianapolis, IN, USA) for 2–3 h until completely separated. Oocytes were then washed

5 times in 1 mg ml^{-1} BSA in Barth's medium. Oocytes were injected with 50 nl (1.1 ng nl^{-1}) of HvSUT1 cRNA and incubated at 15°C in Barth's medium supplemented with 10 μg ml^{-1} gentamycin. Electrophysiological experiments were performed 2–5 days following RNA injection.

Electrophysiological methods

Oocytes were bathed in modified sodium Ringer solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $NaHCO_3$, 10 mM HEPES, 10 mM $MgCl_2$) with continuous perfusion at 1ml min^{-1}. Recording solution pH was 5.0 unless otherwise indicated in figure legends. Recording pipettes, filled with 1 M KCl, with resistances between 1.5 and 5 megaohms were used. Currents were measured using the two-electrode voltage clamp technique with a Dagan TEV 200A amplifier (Dagan Corporation, Minneapolis, MN, USA). Currents were filtered on line at 200 Hz and digitized at 2,000 Hz using pClamp 5.5.1 (Axon Instruments, Inc., Union City, CA, USA). Holding potential was –40 mV and voltage pulses from –157 mV to 57 mV were applied for 150 ms. Steady-state currents are presented as the mean current between 97 and 147 ms following the onset of voltage pulses. Substrate-dependent currents were obtained by subtracting an average of background currents before and after substrate application.

Data analysis

Voltage dependence of $K_{0.5}$ was fitted to the following equation:

$$K_{0.5}(V) = K_{0.5}(0) \times \exp(\delta \times e \times V/(k \times T)) \quad \text{Equation 1}$$

where V is the membrane potential, δ is the fractional electrical distance of the binding site within the membrane electric field, e is the elementary charge, k is Boltzmann's constant, and T is the temperature in Kelvin.

Acknowledgments

This work was supported by the United States Department of Energy Grant DE-FG02–03ER15414 (to JMW). We thank Dr. Winifred Weschke (Gatersleben, Germany) for the plasmid pBK-CMV-HvSUT1. A.S. gratefully acknowledges support from a Bernard and Jean Phinney Graduate Fellowship in Plant Molecular Biology.

References

Aoki, N., Hirose, T., Scofield, G.N., Whitfeld, P.R. and Furbank, R.T. (2003) The sucrose transporter gene family in rice. *Plant Cell Physiol.* 44: 223–232.

Barker, L., Kühn, C., Weise, A., Schulz, A., Gebhardt, C., Hirner, B., Hellmann, H., Schulze, W., Ward, J.M. and Frommer, W.B. (2000) SUT2, a putative sucrose sensor in sieve elements. *Plant Cell.* 12: 1153–1164.

Barth, I., Meyer, S. and Sauer, N. (2003) PmSUC3: characterization of a SUT2/SUC3-type sucrose transporter from *Plantago major*. *Plant Cell.* 15: 1375–1385.

Boorer, K.J., Loo, D.D.F., Frommer, W.B. and Wright, E.M. (1996) Transport mechanism of the cloned potato H^+/sucrose cotransporter StSUT1. *J. Biol. Chem.* 271: 25139–25144.

Carpaneto, A., Geiger, D., Bamberg, E., Sauer, N., Fromm, J. and Hedrich, R. (2005) Phloem-localized, proton-coupled sucrose carrier ZmSUT1 mediates sucrose efflux under control of sucrose gradient and pmf. *J. Biol. Chem.* 280: 21437–21443.

Chandran, D., Reinders, A. and Ward, J.M. (2003) Substrate specificity of the *Arabidopsis thaliana* sucrose transporter AtSUC2. *J. Biol. Chem.* 278: 44320–44325.

Gottwald, J.R., Krysan, P.J., Young, J.C., Evert, R.F. and Sussman, M.R. (2000) Genetic evidence for the *in planta* role of phloem-specific plasma membrane sucrose transporters. *Proc. Natl Acad. Sci. USA* 97: 13979–13984.

Hitz, W.D., Card, P.J. and Ripp, K.G. (1986) Substrate recognition by a sucrose transporting protein. *J. Biol. Chem.* 261: 11986–11991.

Ishimaru, K., Hirose, T., Aoki, N., Takahashi, S., Ono, K., et al. (2001) Antisense expression of a rice sucrose transporter OsSUT1 in rice (*Oryza sativa* L.). *Plant Cell Physiol.* 42: 1181–1185.

Ludewig, U., von Wiren, N. and Frommer, W.B. (2002) Uniport of NH_4^+ by the root hair plasma membrane ammonium transporter LeAMT1;1. *J. Biol. Chem.* 277: 13548–13555.

Meyer, S., Melzer, M., Truernit, E., Hümmer, C., Besenbeck, R., Stadler, R. and Sauer, N. (2000) AtSUC3, a gene encoding a new *Arabidopsis* sucrose transporter, is expressed in cells adjacent to the vascular tissue and in a carpel cell layer. *Plant J.* 24: 869–882.

Patrick, J.W. and Offler, C.E. (2001) Compartmentation of transport and transfer events in developing seeds. *J. Exp. Bot.* 52: 551–564.

Reinders, A. and Ward, J.M. (2001) Functional characterization of the alpha-glucoside transporter Sut1p from *Schizosaccharomyces pombe*, the first fungal homologue of plant sucrose transporters. *Mol. Microbiol.* 39: 445–454.

Riesmeier, J.W., Willmitzer, L. and Frommer, W.B. (1992) Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast. *EMBO J.* 11: 4705–4713.

Sauer, N. and Stolz, J. (1994) SUC1 and SUC2: two sucrose transporters from *Arabidopsis thaliana*; expression and characterization in baker's yeast and identification of the histidine-tagged protein. *Plant J.* 6: 67–77.

Schulze, W., Weise, A., Frommer, W.B. and Ward, J.M. (2000) Function of the cytosolic N-terminus of sucrose transporter AtSUT2 in substrate affinity. *FEBS Lett.* 485: 189–194.

Scofield, G.N., Hirose, T., Gaudron, J.A., Upadhyaya, N.M., Ohsugi, R. and Furbank, R.T. (2002) Antisense suppression of the rice sucrose transporter gene, OsSUT1, leads to impaired grain filling and germination but does not affect photosynthesis. *Funct. Plant Biol.* 29: 815–826.

Truernit, E. (2001) Plant physiology: the importance of sucrose transporters. *Curr. Biol.* 11: R169–171.

Weise, A., Barker, L., Kühn, C., Lalonde, S., Buschmann, H., Frommer, W.B. and Ward J.M. (2000) A new subfamily of sucrose transporters, SUT4, with low affinity/high capacity localized in enucleate sieve elements of plants. *Plant Cell* 12: 1345–1355.

Weschke, W., Panitz, R., Sauer, N., Wang, Q., Neubohn, B., Weber, H. and Wobus, U. (2000) Sucrose transport into barley seeds: molecular characterization of two transporters and implications for seed development and starch accumulation. *Plant J.* 21: 455–467.

Zhou, J.J., Theodoulou, F., Sauer, N., Sanders, D. and Miller, A.J. (1997) A kinetic model with ordered cytoplasmic dissociation for SUC1, an *Arabidopsis* H^+/sucrose cotransporter expressed in *Xenopus* oocytes. *J. Membr. Biol.* 159: 113–125.

(Received March 3, 2005; Accepted July 26, 2005)